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STUDY THE ANTIBACTERIAL ACTIVITY OF COPPER NANOPARTICLES SYNTHESIZED USING HERBAL PLANTS LEAF EXTRACTS

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ABSTRACT

The plants *Asparagus adscendens, Bacopa monnieri, Ocimum bacilicum,* and *Withania somnifera* were used and compared for their extracellular synthesis of metallic copper nanoparticles (CuNPs). Stable Cu nanoparticles were formed by treating aqueous solution of $CuSO_4•5H_2O$ with the plant leaf extracts as reducing agent. By treatment of 1mM aqueous solutions of $CuSO_4•5H_2O$ with leaf extract stable CuNPs were formed; the change in color of solution confirm the formation of stable nanoparticles. UV-Visible study revealed qualitative formation of CuNPs and characteristic absorption peak in *Asparagus adscendens, Bacopa monnieri* and *Ocimum bacilicum* leaf extract at the range of 500-700nm but in *Withania somnifera* leaf extract absorption peak of CuNPs is shifted at the range of 500-800nm. These biosynthesized CuNPs were characterized with the help of Fourier transform infrared spectroscopy (FTIR), and Transmission Electron Microscopy (TEM). The involvement of primary and secondary metabolites or possible reducing agent confirmed by FTIR analysis. TEM confirms the formation and the crystalline nature of Copper nanomaterial. This method can be used as effective and environmental friendly technique for the synthesis of Copper nanoparticles using leaf extract of different plants. The antibacterial potentials of the CuNPs were studied and these are shown good antimicrobial activity against Gram positive and Gram negative bacteria.

KEYWORDS: Copper Nanoparticles, *Asparagus adscendens, Bacopa monnieri, Ocimum bacilicum, Withania somnifera,* Gram Positive and Gram Negative Bacteria

INTRODUCTION

Recently, metal nanoparticles were used as a fundamental building blocks of nanotechnology; due to their unique chemical, op-tical, magnetical, mechanical, and electric magnetical properties having different applications in the field of agriculture, bio-engineering, medicines, electronics, automobiles, nano-fabrics etc (Vaidyanathan *et al,* 2009). Bio-nanotechnology has provided extensive research emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterials. Using physical and chemical methods these specific size controlled metallic nanoparticles were synthesized; however, these methods employ toxic chemicals as reducing agents, or non-biodegradable stabilizing agents and these methods are time consuming and dangerous for the biological systems (Nadagouda and Varma, 2008). Therefore, there is a growing need to develop environmental friendly processes for nanoparticle synthesis without using toxic chemicals. Nowadays biosynthesis of nanoparticles using plant parts and microorganisms has been proposed as a cost effective and eco-friendly alternative to physical and chemical methods.

Using plants for nanoparticles synthesis can be advantageous over other biological processes because of large availability of plants materials and it eliminates the ease of large scale up and eliminates the process of culture maintaining, and no need to use high pressure, energy, temperature and toxic chemical (Singh *et al,* 2010; Rajeshkumar *et al,* 2012). Plant extracts are mostly used in pharmaceutical preparations and they consist of several constituents are acts as reducing and capping agent in the reduction of metal ions. Noble metal nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains. These unique properties are mainly depends on the size, shape and surface area of nanoparticles (Gupta *et al,* 1998; Pal *et al,* 2007).

Among the metallic nanoparticles copper has been enormously utilized for its stable and catalytic properties. Copper nanoparticles, due to their excellent physical and chemical properties and low cost of preparation, have been of great interest. Copper nanoparticles have wide applications as heat transfer systems (Eastman *et al,* 2001) antimicrobial materials (Guduru *et al,* 2007), super strong materials (Male *et al,* 2004; Kang *et al,* 2007), sensors (Xu *et al,* 2006), and catalysts (Athanassiou *et al,* 2006; Pecharromán *et al,* 2006; Rodriguez *et al,* 2007). Plant mediated synthesis of copper nanoparticles was recently reported by using the extracts of plant parts such as *Syzygium aromaticum* Cloves (Subhankari and Nayak, 2013), plants of *Brassica juncea* Indian mustard, *Medicago sativa* Alfa alfa and *Helianthus annus* Sunflower (Cioffi *et al,* 2005), Cu_2O nanoparticles using Tridax procumbens leaf extract was rerepoted antimicrobial activity against Escherichia coli (Gopalakrishnan *et al,* 2012) and Magnolia leaf extract copper nanoparticles also shows antibacterial activity against bacteria (Lee *et al,* 2011).

In this work we are using leaf extract of *Asparagus adscendens* (Shatavari), *Bacopa monnieri* (Brahami), *Ocimum bacilicum* (Tulsi), and *Withania somnifera* (Ashavgandha) plants for synthesis of copper nanoparticles at room temperature. Copper nanoparticles synthesis was identified by color change and UV- visible spectroscopy (UV–vis). The involvement of proteins and possible reducing agent was confirmed by Fourier transform infrared spectroscopy (FTIR), Morphology and composition of copper nanoparticles was characterized by High resolution transmission electron microscopy (HRTEM). Furthermore, the bacterial effect of Copper nanoparticles was also analyzed with gram positive and gram negative microorganisms.

Some steroidal saponins, glycosides and several lipids were reported from roots and leaves (Sharma *et al,* 1982; Sharma and Sharma, 1984; Tandon *et al,* 1990) of *Asparagus adscendens.* Gold nanoparticles as well as platinum and palladium nanoparticles are synthesized from the *Asparagus racemosus* leaf extract (Pandey *et al,* 2012; Raut *et al,* 2013). The known compounds present in *Bacopa monnieri* are triterpenoid saponins known as bacosides, or pseudo-jujubogenin moieties as aglycone units (Chakravarty *et al,* 2003; Sivaramakrishna *et al,* 2005; Bhandari *et al,* 2009) as well as alkaloids brahmine, nicotine, and herpestine have been catalogued, along with D-mannitol, apigenin, hersaponin, monnierasides I–III, cucurbitacins and plantainoside B (Chatterji *et al,* 1963; Chakravarty *et al,* 2002; Bhandari *et al,* 2007). The green synthesis of gold or silver nanoparticles and its antibacterial activity using UV irradiation and leaf extract of *Bacopa monnieri.* (Mahitha *et al,* 2011; Krishnaraj *et al,* 2012; Babu *et al,* 2013) The constituent of *Ocimum* are alkaloids, glycosides, 1,8-cineole, Bergamotene, Eugenol, Linalool, Methyl chavicol compounds (Baritaux *et al,* 1992; Johnson *et al,* 1999; Klimánková *et al,* 2008). Compounds present in basil oil have potent antioxidant, antiviral, and antimicrobial properties, and potential for use in treating cancer (Bozin *et al,* 2006; de Almeida *et al,* 2007). Recently

Ocimum sanctum leaf extracts have been used in the synthesis of silver, gold and platinum nanoparticles (Philip and Unni, 2011; Singhal *et al*, 2011; Soundarrajan *et al*, 2012). The main chemical constituents of *Withania somnifera* are alkaloids and steroidal lactones. Which include tropineand, cuscohygrine and withanolides, isolated from the plant (Ghosal *et al*, 1989; Jayaprakasam *et al*, 2003; Choudhary *et al*, 2004; Mirjalili *et al*, 2009). Biosynthesis of silver nanoparticles using *Withania somnifera* leaf extract and its antibacterial activity reported recently (Patil, 2013; Subbaiah and Savithramma, 2013; Raut *et al*, 2014). Medicinal uses of herbal plants which are used in CuNPs syntheses shows in Table 1.

MATERIALS AND METHODS

Plant Collection

Four medicinal plants, such as *Asparagus adscendens, Bacopa monnieri, Ocimum bacilicum* and *Withania somnifera* were collected from the University campus Figure 1(a-d) respectively. Primarily they were thoroughly washed with distilled water to remove dirt particles. Cleaned herbal parts (leaves) were dried with water absorbent paper (filter paper). 10g chopped leaves of every plant was dispensed in 100 ml of distilled water and boiled for 15 min. at 95ºC using water bath. The suspended mass was then filtered out through Whatman filter paper no.1; the obtained liquid contains the bioactive components leached from the leaves which used further for the synthesis of copper nanoparticles.

Synthesis of Copper Nanoparticles (Medicinal Plants Mediated)

1mM aqueous $CuSO_4$•$5H_2O$ solution were prepared and stored in brown bottles. 10 ml of herbal extracts was taken in conical flask separately and to this drop wise 90 ml of 1m $CuSO_4$•$5H_2O$ solution was added. The same protocol was followed for all the four herbal extracts. The conical flasks were incubated at room temperature. The color change from brown to green was checked periodically (1hr, 16hr, 24hr and 40hr). In Figure 2(a-d) Tube A contains *Asparagus adscendens* leaf extract, *Bacopa monnieri* leaf extract, *Ocimum bacilicum* leaf extract and *Withania somnifera* leaf extract respectively, Tube B contains 1m $CuSO_4$•$5H_2O$ solution and Tube C contain copper nanoparticles solution formed by bioactive components. The change in color visually indicates the formation of copper Nanoparticles (CuNPs) which were used for further characterization study.

UV-Vis Spectra Analysis

The synthesized phytonanoparticles were characterized by UV-Vis spectroscopy, which are widely used for characterization of nanoparticles for the detection of Surface Plasmon Resonance property (SPR) of copper nanoparticles. The aliquots were diluted with double distilled water and spectrum of diluted solution recorded by using UV-Visible spectrophotometer [Model- Shimadzu UV 1800] in the wavelength range 300-800 nm. Baseline correction was made with double distilled water.

FTIR Observations

To remove any free biomass residue or compound that is not the capping ligand of the copper nanoparticles, the residual solution of 100 ml were centrifuged at 10000 rpm for 10 min and the resulting suspension were re-dispersed in 20 ml sterile distilled water. The whole process was repeated three times. Thereafter, the purified suspension freeze dried to obtain dried powder. Finally, the dried copper nanoparticles were analyzed by FTIR. Samples were measured by Bruker Tensor 27 FTIR spectrometer in attenuated total reflection mode (Pike Technologies, Gladi ATR for FTIR with diamond crystal) and using spectral range of 4000-600 cm^{-1}.

High Resonance Transmission Electron Microscopy (HRTEM) and Selected Area Electron Diffraction Measurements (SEAD)

High Resolution Transmission electron microscope (HRTEM) performed for characterizing size and shape of biosynthesized copper nanoparticles. The sample was first sonicated (Vibronics VS 80) for 15 min. A drop of the refined copper nanoparticle solution on the carbon coated copper grid followed by drying under Infrared light for 30 min. HRTEM and SAED measurements were then performed on instrument operated at an accelerating voltage of 200 Kv (Philips; Model CM 200).

Antibacterial Screening

Antibacterial activity were assayed by using standard well diffusion method against human pathogenic Gram positive bacteria *Bacillus subtilis, Staphylococcus aureus* and Gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli.* Bacterial strains grown on nutrient agar at 37°C for 24 hr were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standard (10^8 CFU/ml). Mueller–Hinton agar media was used to perform sensitivity assay (Wagner, 1996). Fresh overnight grown cultures of the bacteria were spread on Nutrient Agar containing Petri plates and with a sterile borer 1mm holes were punched in the medium. 50µl of the solution containing nanoparticles was inoculated in this hole and the plates were incubated at 37°C for 24 hr and the diameter of zone of inhibition produced by the extract compared with those produced by the commercial control antibiotics Streptomycin (25µg/ml).

RESULTS AND DISCUSSIONS

UV-Visible Spectroscopy

Samples of the reaction mixture were diluted with double distilled water and then subjected to the spectral measurement. After mixing the solution of copper sulphate with aqueous leaf extract, the reaction mixture changed rapidly its color. The different color were ascribed formation of copper nanoparticles due to the excitation of surface plasmon vibrations, which indicates the formation of copper nanoparticles directly. Figure 3(a-d) shows the absorption spectra of reaction mixtures of *Asparagus adscendens, Bacopa monnieri, Ocimum bacilicum* and *Withania somnifera* leaf extracts containing copper nanoparticle solution after 1hr, 16hr, 24hr and 40 hr respectively. After the reduction of the ions absorption at 500 to 700 nm range wavelength confirms that the ions of copper sulphate are reduced to copper nanoparticles (CuNPs).

FTIR Analysis

The analysis of FTIR spectra gives an idea about biomolecules bearing different functional groups which are present in underlying system of four herbal plants *Asparagus adscen dens, Bacopa monnieri, Ocimum bacilicum* and *Withania somnifera* leaf extracts containing copper nanoparticles. Comparison between spectra of such leaf extract solutions reveals that *Asparagus adscendens,* and *Withania somnifera* leaf extracts shows Figure 4(a) almost same positions as well as on the magnitude of the absorption bands. On the other hand *Bacopa monnieri,* and *Ocimum bacilicum* leaf extracts also have same absorption bands Figure 4(b)

Figure 4(a) display the FTIR spectrum of the *Asparagus adscendens* leaf extract and copper nanoparticles solution bands at 3260, 2125, 1640, 1082 cm^{-1}. The intense band absorbance at 3260 cm^{-1} is the characteristic of the OH functional group in alcohols and phenolic compounds and the band at 1640 cm^{-1} assigned to the amide I band of the proteins or to

C=C groups/aromatic rings. In addition some weak bands centered at 1082 and 2125 cm^{-1} characteristic to the amide III and amide I band. Whereas the FTIR spectrum obtained from *Withania somnifera* leaf extract and copper nanoparticles solution displays a number of absorption peaks, and presence of multiple bands at 3380, 3210, 2055, 1660, 1580, 1079 cm^{-1} The intense absorbance at 3380 cm^{-1} and 3210 cm^{-1} shows the N-H stretch of amide group and the band at 2055 cm^{-1} assigned to the C≡C stretch of alkynes. The band at 1660 cm^{-1} assigned to the amide I band of the proteins and band at 1580 cm^{-1} demonstrate C-H stretch of primary alcohols, and the band at 1050 cm^{-1} assigned to the C-O stretch of polyols present in the biomass. Thus it is obvious that bands are assigning to the carbonyl groups and secondary amines, are surrounded by some proteins and metabolites having functional groups of amines, alcohols, phenols, ketones, aldehydes, and carboxylic acids.

Figure 4(b) shows the FTIR spectrum obtained from *Bacopa monnieri,* leaf extract and copper nanoparticles solution displays a number of absorption peaks, reflecting its complex nature and shows the multiple bands at 3320, 3301, 2180, 1637, 1372, 1022cm^{-1}. which clearly demonstrates the N-H stretch of amide group at band 3301cm^{-1} and 3320 cm^{-1}, and the C≡C stretch of alkynes at band 2180 cm^{-1}, and C-H stretch of primary alcohols and aromatic groups at band 1637 cm^{-1} and 1599 cm^{-1}, and the presence of CH_3 bend corresponding to alkanes at band 1372 $cm^{-1}$and C-O stretch of polyols in addition some weaker bands centered at 1022cm^{-1}. Whereas the FTIR spectrum absorption peaks of *Ocimum bacilicum* leaf extract and copper nanoparticles solution, shows the presence of multiple bands at 3242, 2061, 2043, 1596, 1050 cm^{-1}. which assigned the N-H stretch of amide group at band 3242 cm^{-1}, and the C≡C stretch of alkynes at band 2061 cm^{-1} and 2043 cm^{-1}, and C-H stretch of primary alcohols at band 1596 cm^{-1}, and C-O stretch of polyols such as flavones, terpenoids and the polysaccharides present in the biomass at 1050 cm^{-1}. Therefore it appears more likely that the reduction of copper ions and stabilization of synthesized copper nanoparticles is the responsibility of many functional groups, including amines, alcohols, ketones, aldehydes, alkenes and carboxylic acids, that are present as plant metabolites and reducing sugars of *Bacopa monnieri, Ocimum bacilicum* herbal plants.

High Resolution Transmission Electron Microscopy (HRTEM)

The microstructures and size of the biosynthesized copper nanoparticles were studied by using HRTEM (High Resolution Transmission Electron Microscopy) analysis. The typical HRTEM images of the copper nanoparticles were synthesized by *Asparagus adscendens* leaf extract as reducing agent is shown in Figure 5(a-b). The micrograph shows formation of spherical-shaped particles in the range of 10–15 nm at 50 nm and 20 nm scale and the insets show the facets. The size is not homogeneous and they are broadly distributed. The insets in Figure 5(a) represent the SAED of these metal particles, which reveals the diffusive ring patterns and attributed to the small particle size. Figure 6(a-b) display the micrograph of biosynthesized copper nanoparticles extract by *Bacopa monnieri* leaf at 50nm and 200nm scale. The particles showed the spherical morphology and the size ranges from 50 to 60 nm. It also shows the leaf extract bounded with the nanoparticles as capping agents to inder further oxidation of nanoparticles. It was observed that the CuNPs possess uniform size of 20–50 nm, almost spherical shape and uniformly distributed in clusters. The insets in Figure 6(a) represent the SAED of these metal particles, and shows the diffusive ring patterns and small particle size. Figure 7(a-b) shows the micrograph of copper nanoparticles synthesized by *Occimum basilicum* leaf extract at 100nm and 200nm scale and the insets show the facets. The size of the particles ranges from 40 to 60 nm and the particles showed spherical morphology. It was observed that the CuNPs possess spherical size and uniformly distributed in clusters. The insets in Figure 7(a) represent the SAED of these metal particles, which reveals the diffusive ring patterns and

attributed to the small particle size. Figure 8(a-b) display the micrograph of biosynthesized copper nanoparticles extract by *Withenia somnifera* leaf at 100nm and 200nm scale. The particles showed spherical shape and the size ranges from 50 to 60 nm. The insets in Figure 8(a) represent the SAED of these metal particles, and shows the diffusive ring patterns and particle size. It is known that spherical as well as non-spherical nanoparticles exhibits better physical properties if they are produced small in size, as the antibacterial properties of copper nanoparticles are size dependent.

Antibacterial Activity

Herbal plants *Asparagus adscendens, Bacopa monnieri, Ocimum bacilicum* and *Withania somnifera* leaf extracts containing copper nanoparticles were subjected to antibacterial activity against *(Sa) Staphylococcus aureus, (Bs) Bacillus subtilis, (Ec) Escherichia coli,* and *(Pa) Pseudomonas aeruginosa*; both Gram-positive and Gram-negative bacteria were sensitive to the extracts. In this study, zone of inhibition recorded for various organisms and copper nanoparticle synthesized from *Asparagus adscendens* leaf extract have potent antibacterial activity against *Staphylococcus aureus* (19mm) where as *Bacopa monnieri* leaf extract exhibited significant activity against *Bacillus subtilis* (16mm), *Escherichia coli* (15mm) and *Pseudomonas aeruginosa* (17mm). Activity of leaf extracts of the plant was comparable to that of reference standard drug Streptomycin 25μg/ml. CuNPs synthesized from herbal leaf extracts exhibited good antimicrobial activity and results were tabulated along with figures and graph (Table 2; figure 9-10).

CONCLUSIONS

It is concluded that the herbal plants extracts of leaf are capable of producing copper nanoparticles extracellular which are quite stable in solution. Biosynthesis of copper nanoparticles using a rapid time scales and low cost procedures using environmentally benign natural resources as an alternative to chemical synthesis protocols. Reduction of copper ions by leaf extracts resulted in the formation of stable copper nanoparticles which are spherical in shape and at a core is capable of rendering antimicrobial efficacy and proved to be active against the pathogenic bacterias. Characterization using UV-Vis and HRTEM analyzer were useful in confirming the formation of nanoparticle synthesis and itscomposition and shape. FTIR analysis results evidenced the formation of copper nanoparticles; which can be studied in future to get the molecular and chemical interation of plants metabolites responsible for the formation of nanoparticles. Thus this study opens up a new opportunity of very conveniently synthesizing copper nanoparticles which can be useful in various potential applications in future.

REFERENCES

1. Athanassiou, E.K, Grass, R.N. and Stark, W. J. (2006). Large-scale production of carbon-coated copper nanoparticles for sensor applications. Nanotechnology, 17: 1668.
2. Babu, P. J, Sharma, P, Saranya, S. and Bora, U. (2013). Synthesis of gold nanoparticles using ethonolic leaf extract of< i> Bacopa monnieri</i> and UV irradiation. Materials Letters, 93: 431-434.
3. Baritaux, O, Richard, H, Touche, J. and Derbesy, M. (1992). Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, ocimum basilicum L. Flavour and Fragrance journal, 7: 267-271.
4. Bhandari, P, Kumar, N, Singh, B. and Kaul, V.K. (2007). Cucurbitacins from< i> Bacopa monnieri</i>. Phytochemistry, 68: 1248-1254.

5. Bhandari, P, Kumar, N, Singh, B. and Kaur, I. (2009). Dammarane triterpenoid saponins from Bacopa monnieri. Canadian Journal of Chemistry, 87: 1230-1234.

6. Bozin, B, Mimica-Dukic, N, Simin, N. and Anackov, G. (2006). Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. Journal of agricultural and food chemistry, 54: 1822-1828.

7. Chakravarty, A. K, Garai, S, Masuda, K, Nakane, T. and Kawahara, N. (2003). Bacopasides III-V: three new triterpenoid glycosides from Bacopa monniera. Chemical and pharmaceutical bulletin-tokyo, 51: 215-217.

8. Chakravarty, A. K, Sarkar, T, Nakane, T, Kawahara, N. and Masuda, K. (2002). New phenylethanoid glycosides from Bacopa monniera. Chemical and pharmaceutical bulletin-tokyo, 50: 1616-1618.

9. Chatterji, N, Rastogi, R. and Dhar, M. (1963). Chemical examination of Bacopa monniera Wettst.: Part I-Isolation of chemical constituents.

10. Choudhary, M. I, Yousuf, S, Nawaz, S. A. and Ahmed, S. (2004). Cholinesterase inhibiting withanolides from Withania somnifera. Chemical & pharmaceutical bulletin, 52: 1358-1361.

11. Cioffi, N, Ditaranto, N, Torsi, L, Picca, R, De Giglio, E, Sabbatini, L, Novello, L, Tantillo, G, Bleve-Zacheo, T. and Zambonin, P. (2005). Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films. Analytical and bioanalytical chemistry, 382: 1912-1918.

12. De Almeida, I, Alviano, D. S, Vieira, D. P, Alves, P. B, Blank, A. F, Lopes, A. H. C, Alviano, C. S. and Maria do Socorro, S. R. (2007). Antigiardial activity of Ocimum basilicum essential oil. Parasitology research, 101: 443-452.

13. Eastman, J, Choi, S, Li, S, Yu, W. and Thompson, L. (2001). Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. Applied Physics Letters, 78: 718-720.

14. Ghosal, S, Lal, J, Srivastava, R, Bhattacharya, S.K, Upadhyay, S.N, Jaiswal, A.K. and Chattopadhyay, U. (1989). Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from Withania somnifera. Phytotherapy Research, 3: 201-206.

15. Gopalakrishnan, K, Ramesh, C, Ragunathan, V. and Thamilselvan, M. (2012). Antibacterial activity of Cu 2 O nanoparticles on E. coli synthesized from Tridax procumbens leaf extract and surface coating with polyaniline. Digest J Nanomat Biostruct, 7: 833-839.

16. Guduru, R. K, Murty, K.L, Youssef, K. M, Scattergood, R. O. and Koch, C. C. (2007). Mechanical behavior of nanocrystalline copper. Materials Science and Engineering: A, 463: 14-21.

17. Gupta, A, Maynes, M. and Silver, S. (1998). Effects of halides on plasmid-mediated silver resistance in Escherichia coli. Applied and environmental microbiology, 64: 5042-5045.

18. Jayaprakasam, B, Zhang, Y, Seeram, N. P. and Nair, M. G. (2003). Growth inhibition of human tumor cell lines by withanolides from< i> Withania somnifera</i> leaves. Life Sciences, 74: 125-132.

19. Johnson, C. B, Kirby, J, Naxakis, G. and Pearson, S. (1999). Substantial UV-B-mediated induction of essential oils in sweet basil (< i> Ocimum basilicum</i> L.). Phytochemistry, 51: 507-510.

20. Kang, X, Mai, Z, Zou, X, Cai, P. and Mo, J. (2007). A sensitive nonenzymatic glucose sensor in alkaline media with a copper nanocluster/multiwall carbon nanotube-modified glassy carbon electrode. Analytical biochemistry, 363: 143-150.

21. Klimánková, E, Holadová, K, Hajšlová, J, Čajka, T, Poustka, J. and Koudela, M. (2008). Aroma profiles of five basil (< i> Ocimum basilicum</i> L.) cultivars grown under conventional and organic conditions. Food chemistry, 107: 464-472.

22. Krishnaraj, C, Jagan, E, Ramachandran, R, Abirami, S, Mohan, N. and Kalaichelvan, P. (2012). Effect of biologically synthesized silver nanoparticles on< i> Bacopa monnieri</i>(Linn.) Wettst. plant growth metabolism. Process Biochemistry, 47: 651-658.

23. Lee, H, Lee, G, Jang, N, Yun, J, Song, J. and Kim, B. (2011). Biological synthesis of copper nanoparticles using plant extract. Nanotechnology, 1: 371-374.

24. Mahitha, B, Raju, B. D. P, Dillip, G, Madhukar, C, Reddyb, K, Manoj, L, Priyanka, S, Jayantha, K. and Raoc, N. (2011). Biosynthesis, characterization and antimicrobial studies of AgNPs extract from Bacopa monniera whole plant. Digest Journal of Nanomaterials and Biostructures, 6: 587-594.

25. Male, K. B, Hrapovic, S, Liu, Y, Wang, D. and Luong, J. H. (2004). Electrochemical detection of carbohydrates using copper nanoparticles and carbon nanotubes. Analytica Chimica Acta, 516: 35-41.

26. Mirjalili, M.H, Moyano, E, Bonfill, M, Cusido, R.M. and Palazón, J. (2009). Steroidal lactones from Withania somnifera, an ancient plant for novel medicine. Molecules, 14: 2373-2393.

27. Nadagouda, M. N. and Varma, R. S. (2008). Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chemistry, 10: 859-862.

28. Pal, S, Tak, Y. K. and Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied and environmental microbiology, 73: 1712-1720.

29. Pandey, S, Oza, G, Gupta, A, Shah, R. and Sharon, M. (2012). The possible involvement of Nitrate Reductase from Asparagus racemosus in Biosynthesis of Gold Nanoparticles. European Journal of Experimental Biology, 2: 475-483.

30. Patil, R. (2013). Green Synthesis of Silver Nanoparticles by Withania somnifera and Evaluation of Its Antimicrobial Potential. Journal of Empirical Biology, 1: 38-48.

31. Pecharromán, C, Esteban-Cubillo, A, Montero, I, Moya, J.S, Aguilar, E, Santarén, J. and Alvarez, A. (2006). Monodisperse and Corrosion-Resistant Metallic Nanoparticles Embedded into Sepiolite Particles for Optical and Magnetic Applications. Journal of the American Ceramic Society, 89: 3043-3049.

32. Philip, D. and Unni, C. (2011). Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (< i> Ocimum sanctum</i>) leaf. Physica E: Low-dimensional Systems and Nanostructures, 43: 1318-1322.

33. Rajeshkumar, S, Kannan, C. and Annadurai, G. (2012). Green synthesis of silver nanoparticles using marine brown algae Turbinaria conoides and its antibacterial activity. J. Pharm. Bio. Sci, 3: 502-510.

34. Raut, R. W, Haroon, M, Sana, A, Malghe, Y. S, Nikam, B. T. and Kashid, S. B. (2013). Rapid biosynthesis of platinum and palladium metal nanoparticles using root extract of Asparagus racemosus Linn. Advanced Materials Letters, 4.

35. Raut, R. W, Mendhulkar, V. D. and Kashid, S. B. (2014). Photosensitized synthesis of silver nanoparticles using < i> Withania somnifera</i> leaf powder and silver nitrate. Journal of Photochemistry and Photobiology B: Biology, 132: 45-55.

36. Rodriguez, J. A, Liu, P, Hrbek, J, Evans, J. and Pérez, M. (2007). Water Gas Shift Reaction on Cu and Au Nanoparticles Supported on CeO2 (111) and ZnO (000$\ bar 1$): Intrinsic Activity and Importance of Support Interactions. Angewandte Chemie International Edition, 46: 1329-1332.

37. Sharma, S, Chand, R. and Sati, O. (1982). Steroidal saponins of< i> Asparagus adscendens</i>. Phytochemistry, 21: 2075-2078.

38. Sharma, S. and Sharma, H. (1984). Oligofuro-and spiro-stanosides of< i> Asparagus adscendens</i>. Phytochemistry, 23: 645-648.

39. Singh, A.K, Talat, M, Singh, D. and Srivastava, O. (2010). Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group. Journal of Nanoparticle Research, 12: 1667-1675.

40. Singhal, G, Bhavesh, R, Kasariya, K, Sharma, A.R. and Singh, R.P. (2011). Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research, 13: 2981-2988.

41. Sivaramakrishna, C, Rao, C.V, Trimurtulu, G, Vanisree, M. and Subbaraju, G.V. (2005). Triterpenoid glycosides from< i> Bacopa monnieri</i>. Phytochemistry, 66: 2719-2728.

42. Soundarrajan, C, Sankari, A, Dhandapani, P, Maruthamuthu, S, Ravichandran, S, Sozhan, G. and Palaniswamy, N. (2012). Rapid biological synthesis of platinum nanoparticles using Ocimum sanctum for water electrolysis applications. Bioprocess and biosystems engineering, 35: 827-833.

43. Subbaiah, K.V. and Savithramma, N. (2013). Antimicrobial Efficacy of Silver Nanoparticles Synthesized from Withania somnifera-An Important Ethnomedicinal Herb of Kurnool District, Andhra Pradesh, India. International Journal of Pharmaceutical Sciences Review & Research, 22.

44. Subhankari, I. and Nayak, P. (2013). Synthesis of Copper Nanoparticles Using Syzygium aromaticum (Cloves) Aqueous Extract by Using Green Chemistry. World, 2: 14-17.

45. Tandon, M, Shukla, Y.N. and Thakur, R.S. (1990). Steroid glycosides from< i> Asparagus adscendens</i>. Phytochemistry, 29: 2957-2959.

46. Vaidyanathan, R, Kalishwaralal, K, Gopalram, S. and Gurunathan, S. (2009). RETRACTED: Nanosilver—The burgeoning therapeutic molecule and its green synthesis. Biotechnology Advances, 27: 924-937.

47. Wagner, H. (1996). Plant drug analysis: a thin layer chromatography atlas. Springer.

48. Xu, Q, Zhao, Y, Xu, J.Z. and Zhu, J.-J. (2006). Preparation of functionalized copper nanoparticles and fabrication of a glucose sensor. Sensors and Actuators B: Chemical, 114: 379-386.

APPENDICES

Figure Captions

Table 1 List of Herbal Plants Collected for Copper Nanoparticle (Cunps) Synthesis.

Table 2 Antibacterial Activity Copper Nanoparticles (Cunps) Synthesized From Herbal Leaf Extracts.

Figure 1 (A) *Asparagus Adscendens,* (B) *Bacopa Monnieri,* (C) *Ocimum Bacilicum,* (D) *Withania Somnifera.*

Figure 2 (A) Tube A- Contain *Asparagus Adscendens* Leaf Extract,Tube B- Contain Copper Sulphate Solution, Tube C- Contain Light Green Colored Copper Nanoparticles Solution, (B) Tube A- Contain *Bacopa Monnieri* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Green Colored Copper Nanoparticles Solution, (C) Tube A- Contain *Ocimum Bacilicum* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Green Colored Copper Nanoparticles Solution, (D) Tube A- Contain *Withania Somnifera* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Light Green Colored Copper Nanoparticles Solution.

Figure 3 (A) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Asparagus Adscendens* Leaf Extract, (B) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Bacopa Monnieri* Leaf Extract, (C) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Ocimum Bacilicum* Leaf Extract, (D) UV-Visible Absorbance Spectra of Copper Nanoparticles With *Withania Somnifera* Leaf Extract.

Figure 4 (A) FTIR Images of Copper Nanoparticles Synthesised From *Asparagus Adscendens* and *Withenia Somnifera* Leaf Extracts, (B) FTIR Images of Copper Nanoparticles Synthesised from *Bacopa Monnieri* and *Ocimum Bacilicum* Leaf Extracts.

Figure 5 HRTEM Micrograph of the Copper Nanoparticles Synthesized From *Asparagus Adscendens* Leaf Extract: (A) The Scale Bar Correspond to 50 Nm, (Insets: SAED of These Metal Particles, (B) The Scale Bar Correspond to 20 Nm.

Figure 6 HRTEM Micrograph of the Copper Nanoparticles Synthesized From *Bacopa Monnieri* Leaf Extract: (A) The Scale Bar Correspond To 50 Nm, (Insets: SAED of These Metal Particles, (B) The Scale Bar Correspond To 200 Nm.

Figure 7 HRTEM Micrograph of the Copper Nanoparticles Synthesized From *Ocimum Bacilicum* Leaf Extract: (A) The Scale Bar Correspond To 100 Nm, (Insets: SAED of These Metal Particles, (B) The Scale Bar Correspond To 200 Nm.

Figure 8 HRTEM Micrograph of the Copper Nanoparticles Synthesized From *Withenia Somnifera* Leaf Extract: (A) The Scale Bar Correspond To 100 Nm, (Insets: SAED of These Metal Particles, (B) The Scale Bar Correspond To 200 Nm.

Figure 9 Zone of Inhibition of Copper Nanoparticles Synthesized from Herbal Leaf Extracts.

Figure 10 Inhibition Zone of Copper Nanoparticles Synthesized from Herbal Leaf Extracts.

Table 1: List of Herbal Plants Collected for Copper Nanoparticle (CuNPs) Synthesis

S. No.	Scientific Name	Common Name	Family	Uses
1.	*Asparagus adscendens*	Shatavari	Asparagaceae	It is a good source of dietary fibre, protein, beta-carotene, vitamin C, vitamin E, vitamin K, riboflavin, phosphorus.
2.	*Bacopa monnieri*	Brahami	Plantagenaceae	It is used in Ayurveda for ulcers, tumors, ascites, indigestion, inflammation and anemia.
3.	*Ocimum bacilicum*	Tulsi	Lamiaceae	It is used in the treatment of headaches, coughs, diarrhea, constipation, worms and kidney malfunctions.
4.	*Withania somnifera*	Ashvgandha	Solanaceae	It is applied externally to tumors, tubercular glands, carbuncles, and ulcers, mental health (anxiety level), fatigue, social functioning, vitality, and overall quality of life.

Table 2: Antibacterial Activity Copper Nanoparticles (CuNPs) Synthesized from Herbal Leaf Extracts

Samples	Leaf Extracts	Inhibition Zone (mm)			
		Sa	Bs	Ec	Pa
Copper nanoparticles	*Asparagus adscendens*	19	13	14	14
	Bacopa monnieri	14	16	15	17
	Ocimum bacilicum	15	13	13	14
	Withania somnifera	13	15	12	15
Standard Antibiotic	Streptomycin	29	27	24	28

Sa *Staphylococcus aureus,* ***Bs*** *Bacillus subtilis,* ***Ec*** *Escherichia coli,* ***Pa*** *Pseudomonas aeruginosa.*

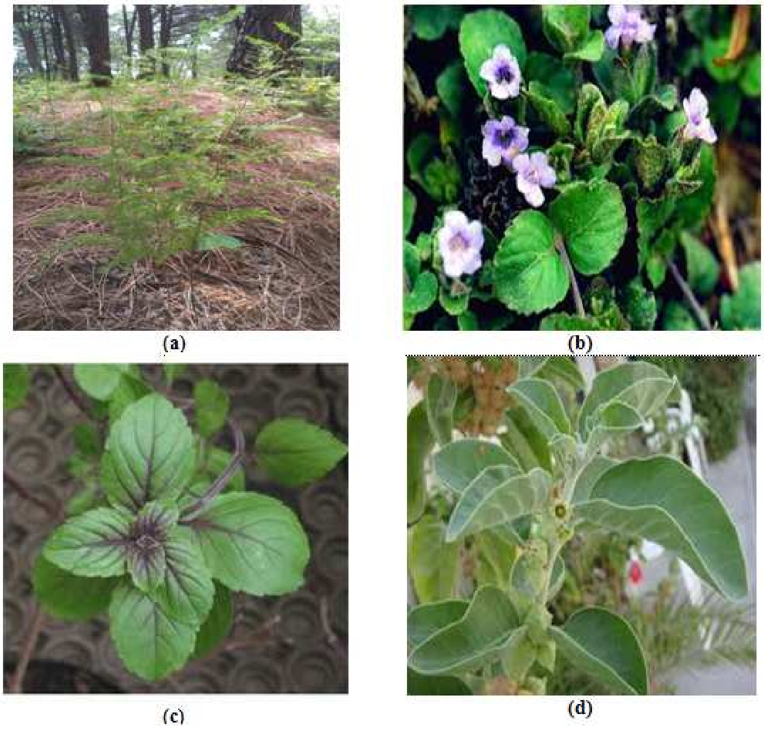


Figure 1: (a) *Asparagus adscendens*, (b) *Bacopa monnieri*, (c) *Ocimum bacilicum*, (d) *Withania somnifera*

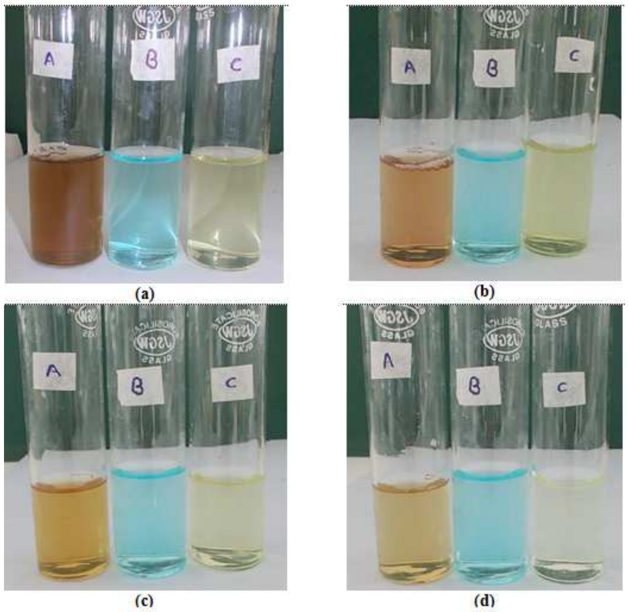


Figure 2: (a) Tube A- Contain *Asparagus adscendens* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Light Green Colored Copper Nanoparticles Solution, (B) Tube A- Contain *Bacopa monnieri* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Green Colored Copper Nanoparticles Solution, (C) Tube A- Contain *Ocimum bacilicum* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Green Colored Copper Nanoparticles Solution, (D) Tube A- Contain *Withania somnifera* Leaf Extract, Tube B- Contain Copper Sulphate Solution, Tube C- Contain Light Green Colored Copper Nanoparticles Solution

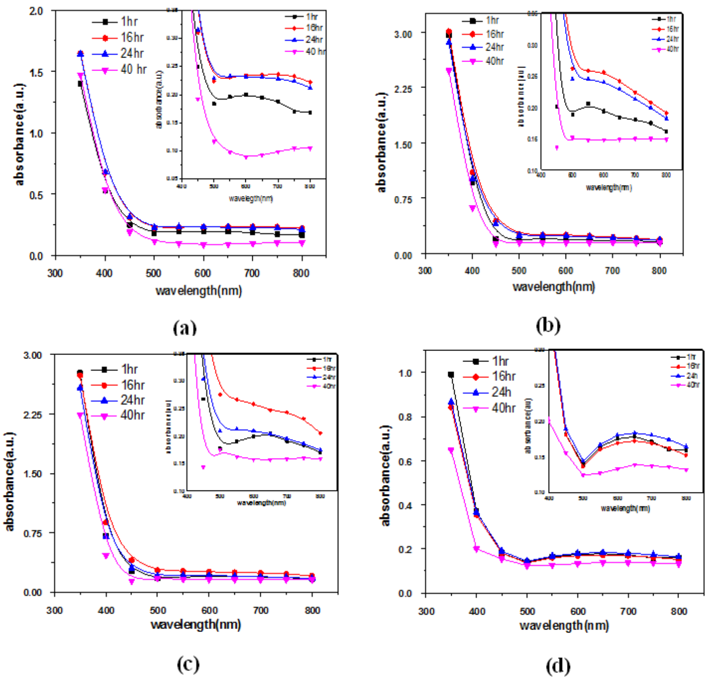
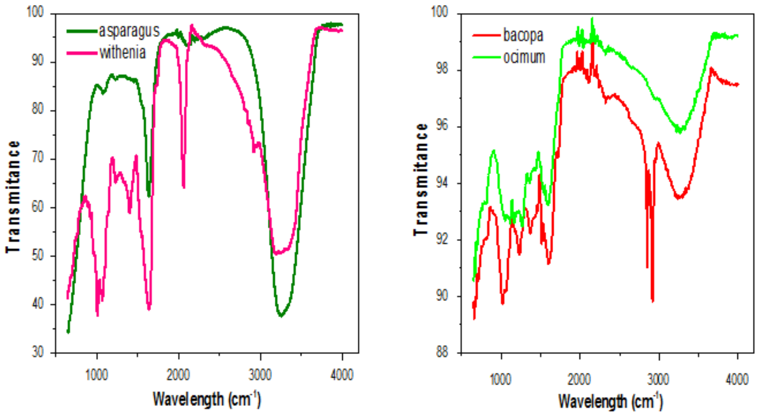
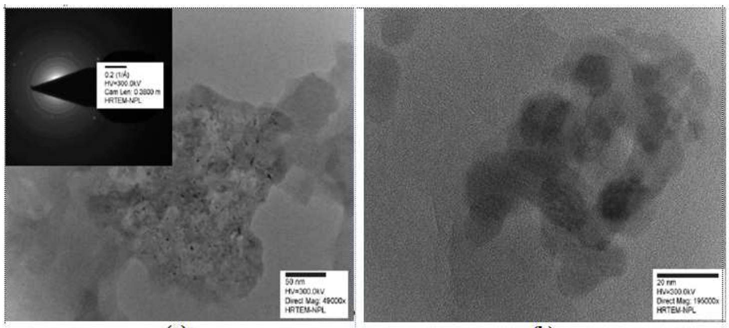


Figure 3: (a) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Asparagus adscendens* Leaf Extract, (b) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Bacopa monnieri* Leaf Extract, (c) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Ocimum bacilicum* Leaf Extract, (d) UV-Visible Absorbance Spectra of Copper Nanoparticles with *Withania somnifera* Leaf Extract



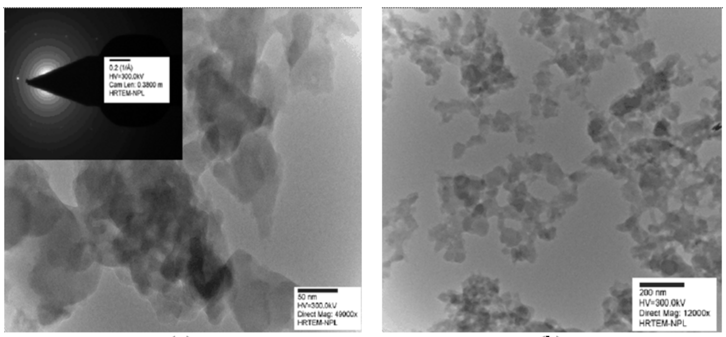
(a) (b)

Figure 4: (a) FTIR Images of Copper Nanoparticles Synthesised from *Asparagus adscendens* and *Withenia somnifera* Leaf Extracts, (b) FTIR Images of Copper Nanoparticles Synthesised from *Bacopa monnieri* and *Ocimum bacilicum* Leaf Extracts



(a) (b)

Figure 5: HRTEM Micrograph of the Copper Nanoparticles Synthesized from *Asparagus adscendens* Leaf Extract: (a) The Scale Bar Correspond to 50 nm, (Insets: SAED of these Metal Particles, (b) The Scale Bar Correspond to 20 nm



(a) (b)

Figure 6: HRTEM Micrograph of the Copper Nanoparticles Synthesized from *Bacopa monnieri* Leaf Extract: (a) The Scale Bar Correspond to 50 nm, (Insets: SAED of these Metal Particles, (b) The Scale Bar Correspond To 200 nm

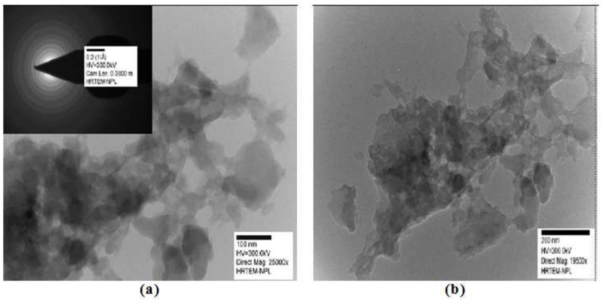


Figure 7: HRTEM Micrograph of the Copper Nanoparticles Synthesized from *Ocimum bacilicum* Leaf Extract: (a) The Scale Bar Correspond to 100 Nm, (Insets: SAED of these Metal Particles, (b) The Scale Bar Correspond to 200 nm

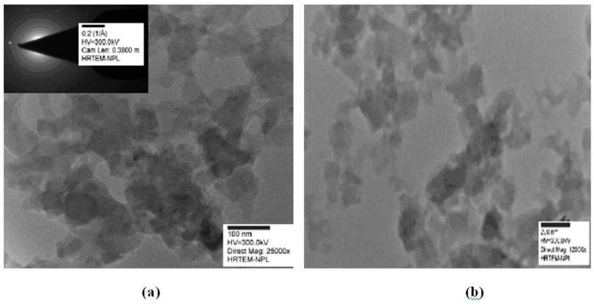


Figure 8: HRTEM Micrograph of the Copper Nanoparticles Synthesized from *Withenia somnifera* Leaf Extract: (a) The Scale Bar Correspond to 100 nm, (Insets: SAED of these Metal Particles, (b) The Scale Bar Correspond to 200 Nm

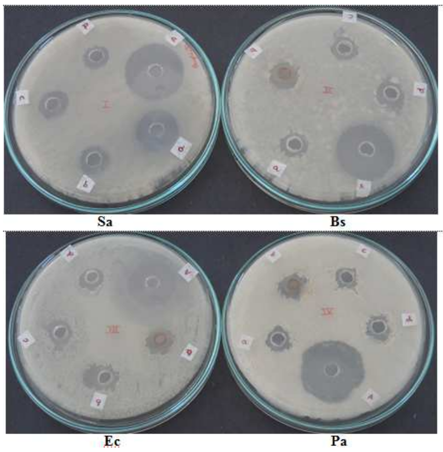


Figure 9: Zone of Inhibition of Copper Nanoparticles Synthesized from Herbal Leaf Extracts

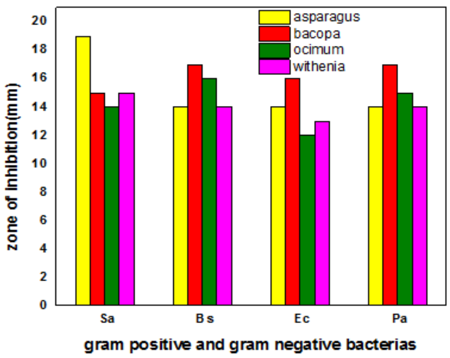


Figure 10: Inhibition Zone of Copper Nanoparticles Synthesized from Herbal Leaf Extracts